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Effects of Prior Land Use, Carbon Availability, and Hydrology on Nitrogen Cycling in Created Freshwater Wetlands

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Effects of Prior Land Use, Carbon Availability, and Hydrology on Nitrogen Cycling in Created Freshwater Wetlands

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ABSTRACT

Freshwater wetlands are a critical feature of the landscape, providing important ecosystem services such as nutrient removal. However, created wetlands often fail to meet functional performance criteria, frequently due to shortcomings in management of key functional drivers, especially hydrology and soil quality. In natural wetlands, hydrology, carbon (C) and nitrogen (N) availability play an important role in nutrient cycling. However, controls on the relative importance of denitrification and N fixation, and release of the greenhouse gas nitrous oxide (N_2O) are poorly understood in created wetlands. The tradeoff between these processes and the mechanisms for improved management are are imperative to achieving functional equivalence. C addition can potentially alleviate the poor organic matter and nutrient levels in created wetlands that limit biogeochemical processes such as denitrification and N fixation. The goal of this project was to determine how hydrological regime and manipulation of organic matter availability affect N cycling in two created wetlands with differing hydrology in Western New York. Hydrological differences between sites significantly influenced potential denitrification, which was significantly higher at the wetter site. The addition of municipal leaf litter compost as a management technique successfully increased soil organic matter, C, N, and moisture content, promoting a 50% increase in potential denitrification without increasing N_2O release. N fixation was not measurable at either site, even with the addition of organic matter. Multiple regression modeling identified different drivers of potential denitrification between sites, with C limiting at the wetter site, and N limiting at the drier site. These results suggest that readily available leaf compost is a viable option to enhance wetland function without also increasing undesirable N_2O emissions, but that simultaneous management of hydrology must occur to ensure maximum N removal.

INTRODUCTION

Wetlands are among the most ecologically and economically valuable ecosystems, providing critically important ecosystem functions and services, such as biodiversity, C storage, erosion control, habitat provisioning, water purification, water cycling, and recreation (Costanza et. al. 1997, Liu et. al. 2010). A key function of inland wetlands is nitrogen (N) removal, which is of enhanced interest in recent years with the increase in harmful algal blooms in freshwater systems (Kaushal et. al. 2011, Watson et. al. 2016, Tian et. al. 2017). Wetlands permanently remove more bioavailable N per unit area than other systems, largely due to saturated soil and high C availability that promote microbial denitrification (Sirivedhin and Gray 2006). In natural wetlands, ecosystem structure and function develop over long periods of time through a series of complex interactions between biotic and abiotic factors. However, more than 50% of wetlands have been lost globally since the beginning of the 20th century, mostly due to human activities (Dahl 1990), leading to an overall loss in this important ecosystem function.

In the early 1990s, as part of the Clean Water Act, the United States passed the "No-Net Loss" policy (Clean Water Act: Section 404) that requires mitigation for unavoidable wetland destruction, with an ultimate goal of maintaining equivalent wetland function. Under this policy, created wetlands are expected to develop key ecosystem functions within approximately 5-10 years (U.S. Army Corps of Engineers 2010). However, created wetlands often do not achieve full functional equivalence within this assessment period, often requiring much longer - up to several decades - to approach similar functionality (Sirivedhin and Gray 2006, Moreno-Mateos 2012). While created wetlands may eventually replicate certain ecosystem functions, like habitat provisioning and biodiversity support (Weisner and Thiere 2010, Jessop et. al. 2015), they often fall short in other areas, such as N removal (Bruland et. al. 2006, Marton et. al. 2014). This shortcoming may exacerbate downstream eutrophication issues, and thereby necessitates a greater understanding of the interactions between abiotic and biotic factors that determine ecosystem function in created wetlands in order to ensure long term wetland sustainability and informed and effective management.

Nitrogen Cycling in Wetlands

N enters a wetland through precipitation, stormwater runoff, groundwater, and the internal process of N fixation (Mitsch and Gosselink 2007, Batson et. al. 2010). N fixation is an anaerobic microbial process mediated by heterotrophic and autotrophic bacteria (and sometimes methanotrophs and sulfate-reducing bacteria) that converts N_2 into biologically available NH_3 for their own use when N is limiting in the environment (Figures 1A and 2). Aerobic ammonification and mineralization convert organic N to ammonium (NH_4^+), which is then oxidized and converted to nitrate (NO_3^-) via the aerobic process of nitrification. While some NO_3^- may be converted to ammonia through the dissimilatory NO_3^- reduction to ammonia (DNRA) pathway, denitrification (DNF) is considered the primary N removal pathway in wetlands (Saunders and Kalff 2001). Anaerobic heterotrophs utilize NO_3^- or NO_2^- as terminal electron acceptors to derive energy from the oxidation of organic matter, producing N_2 as a final product (Figure 1). Globally, this process accounts for the removal of roughly 5.8 teragrams N per year (Jordan et. al. 2011). Under suboptimal oxic conditions for denitrifying microbes, the process can be disrupted, resulting in the release of the potent greenhouse gas nitrous oxide (N_2O) or other intermediate products (NO, NO_2^-) (Knowles 1996, Kampschreur et. al. 2009). Nitrite and ammonium can be reduced under anaerobic conditions to N_2 and water via the anaerobic ammonium oxidation (ANAMMOX) pathway, but this is not well-understood in freshwater wetlands and is typically assumed to be inconsequential relative to denitrification (Zhu et. al. 2010).

Rates of denitrification and N fixation are primarily controlled by a combination of hydrology (Vitousek et. al. 2002, Šantrůčková et. al. 2010, Song et. al. 2010, Racchetti et. al. 2011, Liao and Inglett 2014), N availability (Morris 1991, Vitousek et. al. 2002, Sirivedhin and Gray 2006, Hernandez and Mitsch 2007), C availability (Sirivedhin and Gray 2006, Cohen et. al. 2009,

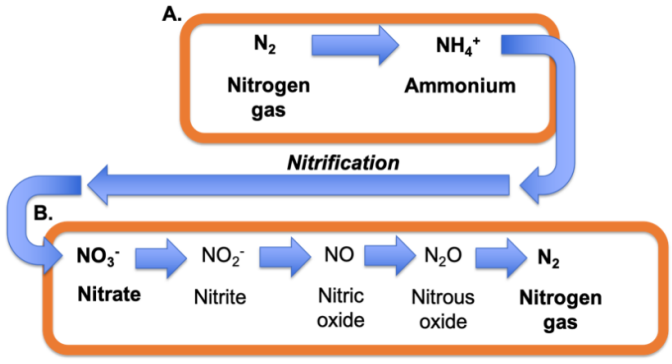

Figure 1. A. Nitrogen fixation, B. Denitrification.

Hopfensperger et. al. 2009, Moseman et. al. 2009, Batson et. al. 2010, Lishawa et. al. 2014, Zhang et. al. 2017, Murphy et. al. 2018) and temperature (Klein et. al. 2017). Some studies have suggested that denitrification and N fixation rates in created wetlands may be significantly lower than in natural counterparts due to unsuitable conditions for both groups of microbes (Sirivedhin and Gray 2006, Moseman et. al. 2009). Similarly, in created wetlands, the release of N_2O may be common, as conditions are often suboptimal due to inadequate hydrological management or high nutrient influxes (Hernandez and Mitsch 2007, Wang et. al. 2014, Lyu et. al. 2017). The balance of these processes has implications for both greenhouse gas emission and permanent removal of N from watersheds. Consequently, estimates of N_2 and N_2O flux, along with N fixation, and an understanding of the primary driving factors, can offer insight for management to promote desirable wetland function.

Table 1. Hypothesized effects of hydrology, represented as long and short hydroperiods, and carbon/nitrogen availability, represented in high and low carbon or nitrogen, on denitrification (DNF), autotrophic nitrogen fixation (NF_{auto}), and heterotrophic nitrogen fixation (NF_{hetero}).

	DNF	NF_{auto}	NF_{hetero}
Long hydroperiod	+	+	+
Short hydroperiod	-	-	-
High nitrogen	+	-	-
Low nitrogen	-	+	+
High carbon	+	-	+
Low carbon	-	+	-

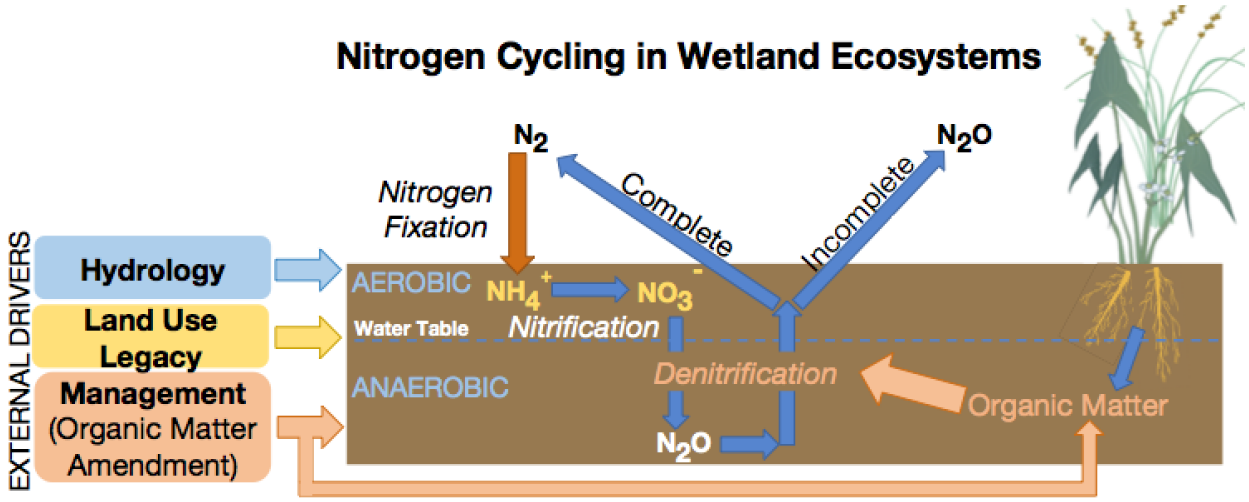

Figure 2. Factors driving nitrogen cycling in wetlands.

Hydrology

Wetland hydrology plays an integral role in determining overall ecosystem function by altering soil properties, biogeochemical cycling, and biotic community structure. In wetlands, the water budget is primarily defined by surface water inputs via streams, runoff, and precipitation, and balanced by outflow and evapotranspiration. Timing and duration of flooding determine oxygen availability in the soil and water, therefore defining the presence and extent of aerobic and anaerobic zones (Vitousek et. al. 2002, Song et. al. 2010, Racchetti et. al. 2011). Due to the anaerobic conditions required for DNF and N fixation, wetland hydrology may be the most important driver of N-cycling by directly determining spatial and temporal structure of anaerobic zones (Figure 2). Denitrification is likely enhanced in created wetlands with longer hydroperiod, provided that sufficient nitrate is available. Under a fluctuating hydroperiod, denitrification can be interrupted due to sudden exposure to aerobic conditions, resulting in N_2O release (Knowles 1996, Kampschreur et. al. 2009), which may be more common in created wetlands subject to erratic hydroperiod. It is not clear, however, how the balance between N_2 and N_2O, or the efficiency of complete denitrification, may recover in the months or years following such a disturbance.

Hydrology may also influence the balance between autotrophic and heterotrophic N fixation (Liao and Inglett 2014). High water levels limit emergent macrophytes, thereby prompting a shift from heterotrophic to autotrophic N-fixers, as heterotrophic fixation is often favored when

macrophytes are dense (Vitousek et. al. 2002, Šantrůčková et. al. 2010). In small, created wetlands, where rapid swings in water level due to drought, lack of water control, or invasion by highly productive plants may occur, I hypothesize that autotrophic N fixation will be low. Further, periods of drying and subsequent oxidation of soils will likely limit heterotrophic N fixation.

Nitrogen Availability

Reactive N in wetlands derives from external inputs including fertilizers and other chemicals that enter through precipitation, groundwater or runoff, internal N-fixation, and mineralization of decomposing wetland plants and other organic material (Batson et. al. 2010; Figure 2). Many studies have illustrated the importance of N availability for the magnitude of both denitrification and N fixation in wetlands (e.g., Morris 1991, Vitousek et. al. 2002, Sirivedhin and Gray 2006, Hernandez and Mitsch 2007). Denitrifying microbes are limited by the availability of NO_3^- for use as a terminal electron acceptor, with higher rates when N is abundant (Sirivedhin and Gray 2006). However, under abundant conditions, N fixing microbes abstain from operating their own energy intensive N fixing process, instead opting to take advantage of freely available N (Vitousek et. al. 2002). Created wetlands tend to have significantly lower soil N than their natural counterparts (e.g., Fennessey et. al. 2008), underscoring the need to take N availability into account when considering drivers of N cycle processes.

Nutrient availability and hydroperiod may be linked in some wetlands. In riverine systems where flood waters bring in nutrients, the frequency of flooding drives denitrification by delivering external nutrients (Hernandez and Mitsch 2007); however, higher nutrient levels may inhibit N-fixers (Kox et. al. 2016), leading to net removal by the microbial community. At the same time, high water levels produced by long hydroperiods may also limit the aerobic process of nitrification, and thereby limit denitrification by diminishing the supply of NO_3^- (Austin et. al. 2019). This balance is challenging to unravel in wetlands that are subject to wet-dry extremes, which may be more common in the small, disconnected wetlands typical of mitigation projects, but is important to the understanding of overall N dynamics in wetlands.

Carbon Availability

Because denitrifying and some N-fixing bacteria are heterotrophic, relying on organic C as the primary electron donor for anaerobic respiration (Batson et. al. 2010), both quantity and quality of organic matter availability drive overall process rates (Sirivedhin and Gray 2006). Previous investigations of the role of C quantity and quality have used the addition of simple sugars like glucose, or alginate (e.g., Sirivedhin and Gray 2006, Cohen et. al. 2009) and typically show a positive increase correlated with increasing lability for both denitrification and N-fixation (Sirivedhin and Gray 2006, Cohen et. al. 2009, Lishawa et. al. 2014, Zhang et. al. 2017, Murphy et. al. 2018). Without a suitably labile source of C, both denitrification and heterotrophic N fixation may cease (Starr and Gillham 1993).

Plants are the primary source of organic matter in wetlands, and plant community composition is important in driving N cycling because the lability of detritus can vary widely across species (Hopfensperger et. al. 2009). Plant community composition and productivity are determined largely by local hydrology, suggesting the existence of a distinct indirect relationship between hydrology and N cycling, with particular relevance to denitrification and N fixation processes. Likewise, plant nutrient uptake and immobilization may compete with heterotrophic microbes (Moore et. al. 2015), limiting the stimulation of denitrification by organic matter production.

With special relevance to created wetlands, which are often more prone to biological invasions (Lishawa et. al. 2014), some studies have suggested that denitrifying and N fixing microbial communities are more vulnerable due to changes in C lability and quantities (Moseman et. al. 2009). Certain species have been found to have potentially significant effects on C quantity and quality, such as *Solidago canadensis* (Canada goldenrod, Ye et. al. 2019) and *Phalaris arundinacea* (reed canary grass, Stefanowicz et al. 2018). Notably, a species like *P. arundinacea* is a noxious invader of wetlands, presenting problems especially in created systems. Herbicide is often used as a means of controlling these invasions, increasing the supply of labile detritus in the short term, but inhibiting new plant growth and subsequent C production in the long term.

In created wetlands with poorly developed soils, low soil organic content may inhibit N cycling. In the interest of improving N removal via denitrification, the use of highly labile C addition as a management strategy in created wetlands has been investigated in small-scale plots (Ballentine and Schneider 2009, Cohen et. al. 2009, Ballentine et. al. 2011, Lishawa et. al. 2014) with promising results. However, the concomitant impact on N fixation and the interaction between enhanced C availability and hydrology remain unknown (Figure 2). Further, most of these methods have evaluated the use of materials such as sugar, or saw dust, that may be impractical on a larger scale and that have a relatively high C:N, so that the auxiliary addition of N is limited. It is therefore uncertain whether large-scale C addition as a management strategy improves both soil characteristics and overall N removal without also enhancing N_2O release.

We currently lack a complete understanding of the relative importance of C availability, N availability, and hydrology in created wetlands for denitrification and N fixation, especially under differing management strategies. Of particular interest is the development of management practices that maximize N removal in created freshwater wetlands in addition to habitat provisioning services without amplifying harmful greenhouse gas emissions. In this work, we evaluate the use of municipal compost on N cycling as a potential management strategy for large-scale soil enhancement in wetlands. The evaluation takes place in two wetlands with similar age and N availability but contrasting hydrology. We hypothesized that: (1) denitrification will increase in response to compost addition, (2) compost addition will stimulate N_2O effluxes in both areas, with a larger increase at the drier site; and (3) N fixation will increase due to added C in both wetlands. The ultimate goal of the work is to provide a better understanding of the relationship between C availability and hydrology in created freshwater wetlands in order to improve N removal without a concomitant increase in greenhouse gas emissions.

METHODS

Project Overview

To study the relative importance of key drivers of N cycling, I took advantage of a long-term C addition experiment in two created wetlands that differed in hydrology. Over three growing seasons from 2017-2019, potential denitrification and N fixation rates were measured *ex situ* and

N_2O fluxes were measured in the field in static chambers that isolated soil and plant plus soil-derived N_2O release. Soil physicochemical properties including temperature, pH, bulk density, nutrients (extractable inorganic N, total N, total P), organic C, plant community composition, and hydrologic regime (soil water content, standing water depth) were measured seasonally. During the study period, precipitation patterns varied substantially from the regional mean resulting in drought conditions, and herbicide was applied for control of *P. arundinacea* (reed canary grass) in one wetland, allowing the opportunity to evaluate resilience to environmental perturbations.

Study Site

This study took place at the High Acres Nature Area (HANA) in Perinton, New York, USA, a private property that consists of approximately 153 hectares of natural and created wetlands, ponds, old fields, and woodlands (Figure 3). Most of the original natural wetlands at HANA were filled in during construction of the Erie Canal and agricultural development in the 1800s. Following decades of use for row crop and livestock agriculture, and gravel mining, the site was purchased by Waste Management of New York (WM) in 1986. Between 2009 and 2012, several wetlands were created on the site to mitigate filling of wetlands in the adjacent landfill, and the site is currently managed for conservation and open to the public year-round for passive recreation.

Two created wetlands were selected for this study, the southern cell of Cady Wetland complex (Mitigation Area 2; 43°05'34.28" N, 77°22'45.31" W) and the southern cell of the Packard Wetland complex (Mitigation Area 3A; 43°05'16.14" N, 77°22'15.06" W; Table 2). The Cady wetland was converted from a row crop agricultural field to a depressional emergent wetland in 2009 and except under very dry conditions (e.g., 2016) had saturated soil or standing water year round (Figure 5). From creation until the fall of 2017, the plant community was dominated by *Typha latifolia*, *Typha angustifolia* (broadleaf and narrowleaf cattail respectively), *Persicaria maculosa* (spotted ladysthumb smartweed), *Lythrum salicaria* (purple loosestrife), and *P. arundinacea*, with increasing cover of *P. arundinacea* after the extreme drought of 2016. In fall 2017, herbicide (glyphosate) was applied to eliminate *P. arundinacea*. The Packard site was converted from cow pasture to a forested-shrub-scrub-wet meadow wetland complex in 2012

(Table 2) and typically dries during late spring. At the time of this experiment, the plant community was representative of wet meadow species, including *Daucus carota* (Queen Anne's Lace), *Solidago canadensis* (Canada goldenrod), *P. pensylvanicum* (Pennsylvania smartweed), and *Typha angustifolia* (narrowleaf cattail), with young trees (*Quercus bicolor* [swamp white oak] and *Acer rubrum* [red maple] on the fringe.

Table 2. Key characteristics of Cady and Packard Wetlands at the High Acres Nature Area in Perinton, NY, USA.

	Creation Date	Prior Land Use	Relative Nutrient Levels	Hydrology	Vegetation Community
A2S	2009	Row Crop Agriculture	Similar	Consistent, low water levels	Low species richness (S), dominated by *Typha spp., Persicaria* sp., and *P. arundinacea*
A3A	2012	Cow Pasture	Similar	Seasonal, low water levels	High species richness (S), dominated by native, herbaceous wet meadow species

Experimental Design

In the spring of 2014 (Cady Wetland) or 2015 (Packard Wetland) a long-term C addition experiment was initiated to evaluate the impact of organic matter amendment on wetland development (Figure 3). At each site, five pairs of 2 x 30 m zones (60 m^2 each) were established and each year in late spring (May-June), municipal leaf litter compost was added to half of the transects to a depth of approximately 5 cm. The C:N molar ratio of compost was ~18.7 C:N (28% C, 1.8% N). Taking into account plant cover, the total area of each plot covered by compost was estimated at 50 +/- 4%, leading to a supplement of roughly 2.0 kg C m^{-2} and 0.13 kg N m^{-2} (Figure 4).

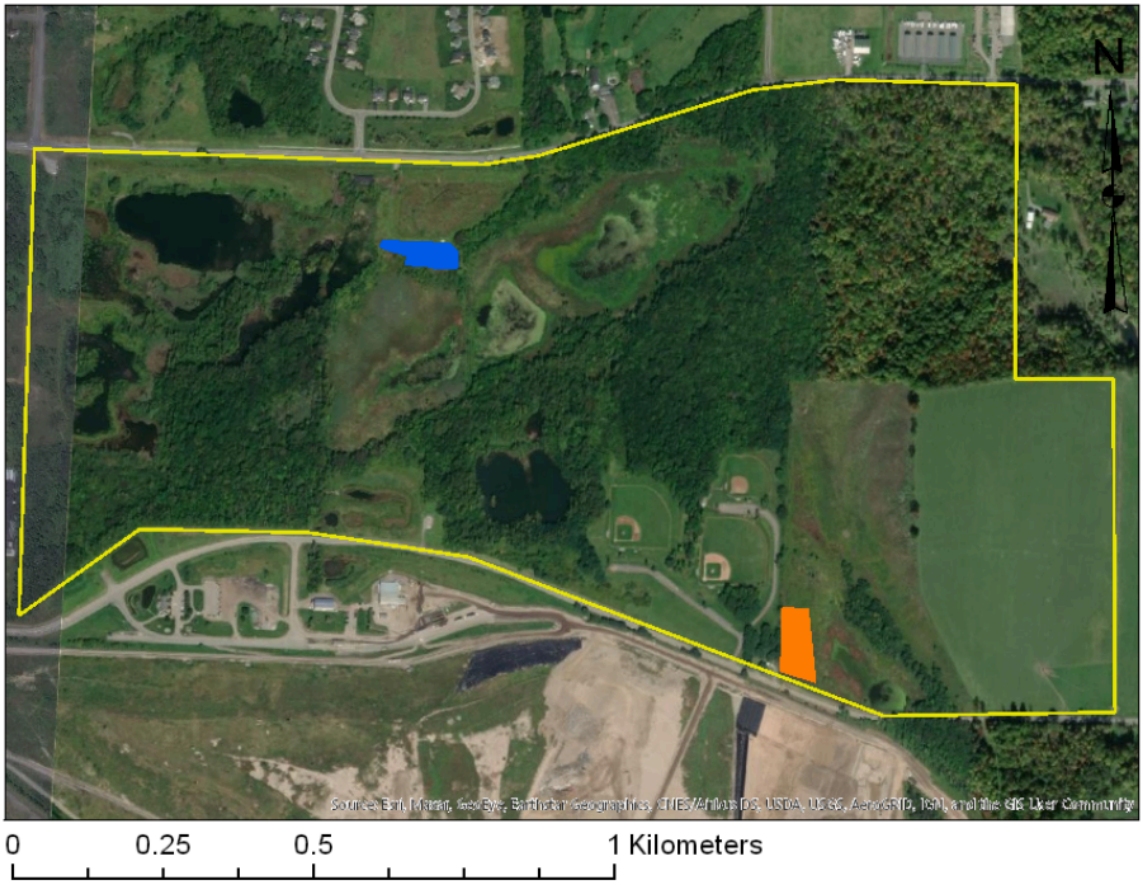

Figure 3. Aerial image of the High Acres Nature Area (HANA) showing Packard (orange, bottom right) and Cady (blue, top left) created wetlands. Image courtesy of Google Earth.

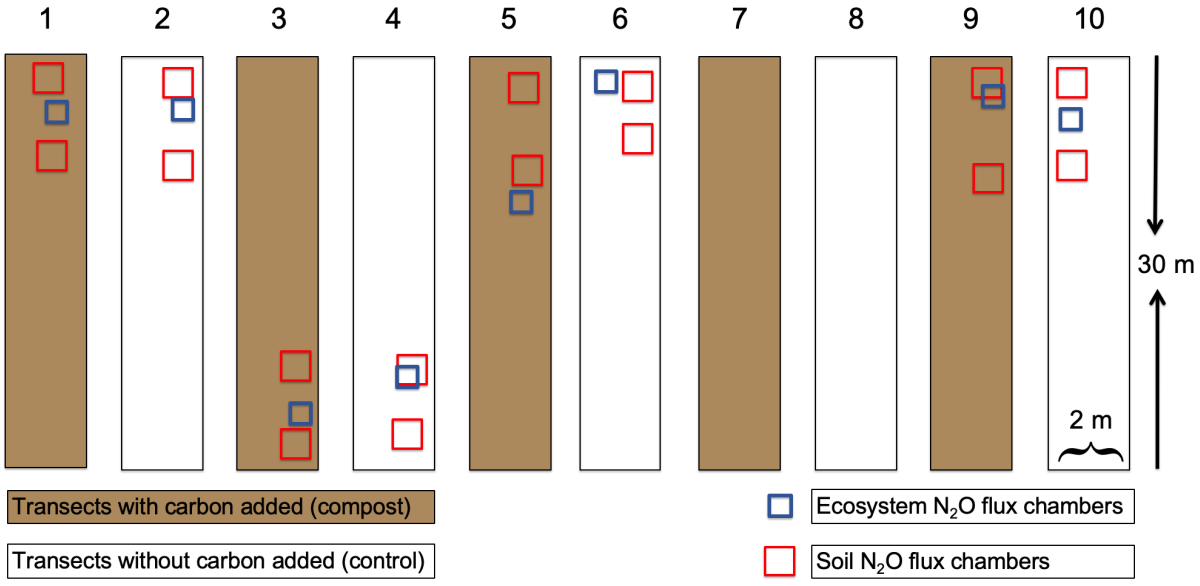

Figure 4. General experimental layout of transects showing paired control and compost addition transects and embedded sampling plots.

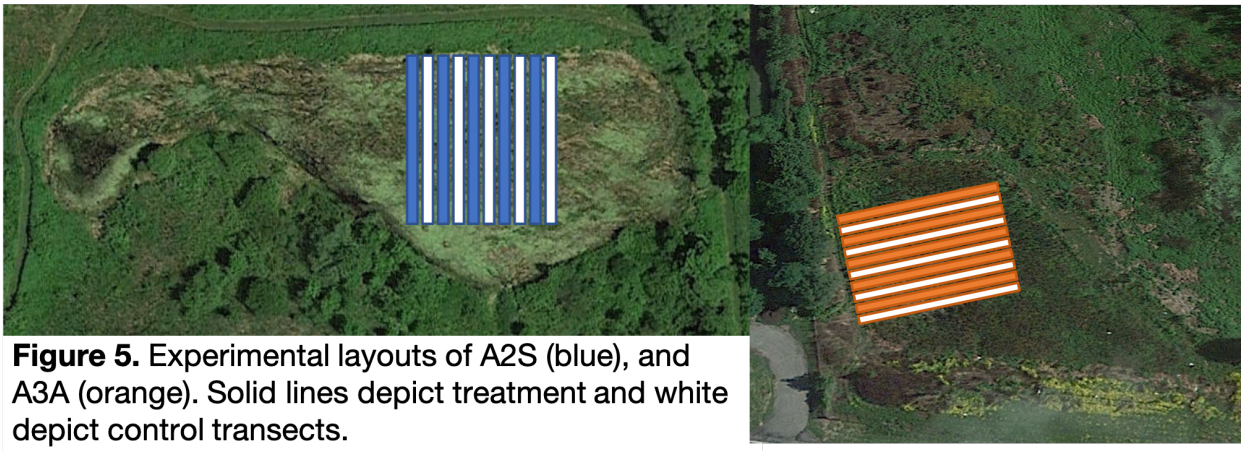

Figure 5. Experimental layouts of A2S (blue), and A3A (orange). Solid lines depict treatment and white depict control transects.

Soil Characterization

Hydrological characterization was based on water depth and precipitation. Standing water depth was measured seasonally beginning in fall 2017 (fall, spring, summer) at three points in each plot (n = 8 plots per transect). Precipitation data was from the Crescent Trail/Aldrich Rd weather station (KNYFAIRP29) in Perinton, NY, and downloaded from Weatherunderground.com. Soil characteristics, including pH, organic matter, moisture content, bulk density, extractable inorganic N, and total N, P and C were evaluated across sites and treatments in summer and fall of 2018 and spring, summer, and fall of 2019. Soil samples (10 cm depth x 6 cm ID) were collected adjacent to soil chamber plots (n = 2 per transect). Soil pH was evaluated by creating a 2:1 deionized water:soil slurry and analyzed using a Hach pH meter in summer 2018 and fall 2019 only. Organic matter content was assessed using the loss-on-combustion method in all 2018 and 2019 collection periods with the exception of fall 2018 in Packard. Soil was weighed, oven-dried at 60 °C for 24 hr and then combusted in a muffle furnace for 4 hr at 550 °C (Heiri et. al. 2001). Using the mass at each step and the initial volume of the sample, moisture content and bulk density were also assessed. A subsample of oven-dried soil was homogenized using an electric coffee mill for elemental analysis.

Percent N and C were measured using a Perkin Elmer 2400 Elemental Analyzer (EA). Total and inorganic P were measured using the ammonium molybdate method (Murphy and Riley 1962) following extraction in 1N HCl. Prior to extraction, soil for total P analysis was treated with a $Mg(NO_3)_2$ solution and combustion at 550 °C for 2 hr. After settling, colorimetric analysis of the supernatant was measured using a Shimadzu 1800 spectrophotometer.

Soil NO_3^- and NH_4^+ were assessed using the KCl extraction method using paired 5 g aliquots of wet soil. The first was used to determine the dry weight and the second was placed into a 50 mL centrifuge tube with 50 mL of 2M KCl and shaken for 30 min on a rotary shaker. The samples were then centrifuged at 5000 rpm for 10 min, filtered (0.45 um Supor), and frozen. NH_4^+ was measured using the phenol-hypochlorite method (Maynard et. al. 2008) and NO_3^- was measured using a vanadium-based method described by Doane and Horwath (2003).

Vegetation Community

Vegetation community composition was measured seasonally in the Spring, Summer, and Fall of 2018 and 2019 in eight permanent vegetation plots per zone. Total cover (% Cover) of each species was assessed in two person teams in the 1 m^2 plots, and species richness (*S*) and Shannon diversity index (*H*') values were calculated.

Denitrification Potential

Denitrification potential (PDNF) was measured *ex situ* using the acetylene block technique, which measures enzymatic activity in the soil under anaerobic conditions (Ryden et. al. 1987, Groffman et. al. 1999). Acetylene blocks the final step in denitrification, the conversion from N_2O to N_2, and is an indirect measure of potential denitrification. Soil samples were collected in late September of 2017, July and October of 2018 (n=8 per treatment per site; transects 7 and 8 excluded; Figure 4), and May, July, and October of 2019 from just outside each small chamber plot (increased replication to include transects 7 and 8; n=10 per treatment per site) to a depth of 10 cm using a tulip bulb auger (6 cm ID), refrigerated overnight, and analyzed within 24 hr of collection. Ten g of sieved soil (#8 mesh, 2.38 mm opening) was added to 125 mL clear jars, and 5 mL of media (nitrate 100 mg^{-1} kg^{-1} + dextrose 40 mg^{-1} kg^{-1}+ chloramphenicol 10 mg^{-1} kg^{-1}) and 5 mL of Nanopure water were added to each jar to alleviate N and C limitation. The jars were

then sealed, crimped, and purged three times for one min intervals with N_2 gas, shaking in between, to degas. Immediately, 12.5 mL acetylene was added, and jars were shaken for another minute and the initial sample was taken with a 20 mL syringe and stored in pre-evacuated crimp top vials. Jars were placed on a rotary shaker in between subsequent sampling points at 0, 30, 60, and 120 min. Samples were analyzed as above.

Nitrogen Fixation Potential

N fixation potential was also measured *ex situ* using the acetylene reduction technique (Reynolds et. al. 2015). Soil samples were collected and prepared as described for potential denitrification. For each plot, 10 g of sieved soil (#8 mesh, 2.38 mm opening) was added to 125 mL clear jars. Artificial freshwater (62.5 mL) was then added to each jar, which were sealed, crimped, and purged three times for one minute intervals with N_2 gas, shaken in between, to remove dissolved gases. Immediately, 10 mL of acetylene was added to the jars before being shaken for another minute. After taking an initial gas sample (T_0), jars were placed on a rotary shaker until the next sampling interval. Samples were taken with a 20 mL syringe at 0, 30, 60, and 120 min and stored for later analysis by GC. Measurements were taken in October of 2018 under lab light conditions only. In May, July, and October of 2019 measurements were made in both light (Caron Environmental Chamber with full spectrum fluorescent bulbs) and in foil wrapped jars to simulate dark to separate potential heterotrophic and autotrophic contributions to N fixation.

Plant and Soil N_2O Fluxes

In order to assess the impact of compost addition on N removal via N_2O, gas fluxes were measured using two closed chamber designs that effectively separate the role of soil microbes and emergent plants (Figure 4). Measurements of soil-only gas fluxes were conducted in May, July, and September of 2017, July and October of 2018, and May, July, and September of 2019 from two permanent plots in each zones, with two replicate chambers per plot. Separate soil-only flux measurements were assessed in September of 2019 to determine the impact of precipitation on fluxes. The two chambers within each plot were averaged, but the two plots in each transect were treated as separate replicates (n=8 per treatment per site; transects 7 and 8 were excluded; Figure 4). In 2017, a PVC coupling (0.115 m ID *0.10m length) was inserted permanently into the soil, level with the soil (Figure 3, red squares). During measurements, a length of

polycarbonate tubing (0.10 m ID* 0.0032 m wall * 0.30 m length) was inserted into the coupling. The tubing had a gas tight acrylic lid with a sampling port inserted into a 1.5 cm diameter hole in the lid. In 2018, the chamber design was altered to enclose a larger soil area. Two sections of 15 cm diameter PVC pipe were inserted permanently in each plot, in the approximate location of 2017 measurements, to a depth of ~4-5 cm. During field measurements of soil N_2O fluxes, a ~18 cm tall PVC end cap was fitted to the top of each semi-permanent base and made airtight by a rubber seal attached to the sides of each chamber. A sampling port consisting of Vincon tubing, a bulkhead, and stopcock, sealed with epoxy, was installed on the top of each chamber. Before placing the chamber on the base, any aboveground vegetation within the base was carefully clipped and removed to isolate soil processes. Gas samples were taken every ten minutes over a 30 min period (4 time points) using a 20 mL syringe and stored in 10 mL crimp top vials for later analysis on a gas chromatograph (Shimadzu 2014 Greenhouse Gas Analyzer). Fluxes were calculated based on the change in concentration over time.

In order to assess the role of both plants and soil on N_2O fluxes, a larger clear chamber that enclosed the plant canopy was used. The chamber featured a cooling and air circulation system to maintain temperature and mix the headspace. Measurements were taken in June and September of 2018 and 2019 from one permanent plot (~0.5 x 0.5 x 1.5m) per zone at both sites (n=4 per treatment at each site). Two bulkheads, located on the side of the chamber, were attached to tubing and a small submersible pump that continuously circulated chilled water through a small radiator mounted inside the chamber. Temperature was continually monitored with a thermometer attached to the interior wall of the chamber and kept as close to ambient as possible by adjusting water circulation. On the top of the chamber, two bulkheads served as input and output for tubing connected to a portable LI-820 infrared CO_2 gas analyzer (IRGA); chamber air was circulated between the outlet and the IRGA with a small air pump. A third bulkhead served as a sampling port for gas collection. To ascertain the role of plants on N_2O fluxes, light and dark conditions were simulated in situ. Dark conditions were created by wrapping each chamber in reflective mylar sheeting. Gas samples were collected in a 20 mL syringe at 15 minute intervals over a 45 min period (4 time points) in the light and then in the dark and stored and analyzed as above. Fluxes were calculated separately for light and dark based on the change in concentration over time.

Statistical Analyses

I completed all statistical analyses using JMP Pro 15.0 statistical software. Prior to analysis, all data were checked for normality and heterogeneity of variance, and where possible transformed to meet these assumptions. Soil properties, plant community characteristics, ecosystem N_2O fluxes measured in the light only, soil N_2O fluxes, and PDNF were assessed for each site individually using two-way analysis of variance with treatment and sampling date as fixed factors in a full factorial design, including interactions (ANOVA) when variables were successfully normalized. When significant date or date x treatment interactions were found, Tukey's HSD post hoc test was used to identify significant differences. Data that were unable to be normalized were analyzed using a Kruskal-Wallace nonparametric test for treatment and season separately, precluding analysis of treatment x season interactions. Potential spatial variation across each site was accounted for by treating adjacent pairs of zones as a single block (n=5 blocks per site) and using this as a random factor in the analysis. To assess between-site differences, a one-way ANOVA was run on control plots only, across all seasonal measurements. To evaluate for the potential impact of plants on N_2O flux in the ecosystem chambers, a paired t-test was conducted between rates in the light and the dark. To determine the effect of ~16 mm of precipitation on soil N_2O fluxes, a second paired t-test was conducted between fluxes before and after rainfall.

Forward regression modeling was conducted to determine best predictors of PDNF and soil N_2O release for 2018-2019. For strongly autocorrelated variables (TP and IP; *S* and *H'*), one variable was eliminated, resulting in the final set of potential predictors: water depth, soil C, soil N, OM, MC, BD, plant cover, *H'*, temperature, and precipitation (calculated based on a sum of the previous ten days before each process measurement) for 2018 and 2019 only. Incomplete data for 2017 precluded inclusion. The best model was selected using Akaike information criterion (AIC). One over-arching model was created for all sites and treatments, and then separate models for each site were created.

In an attempt to assess the role of plant community composition on N cycling, three plant species were selected for a separate forward regression analysis against soil C, total N, and C:N for

2018-2019. Selected species were either found in at least half of all seasons in 15% of the plots with cover >30%, or were deemed potentially important based on the literature. *S. canadensis* met both criteria, with both high abundance and literature that suggested presence might affect soil C concentrations by providing high aboveground biomass and a labile C source (Ye et. al. 2019). *P. arundinacea* met the second, with prior work suggesting an impact on N availability due to efficient plant uptake (Stefanowicz et. al. 2018). The smartweeds, *P. pensylvanicum* (Packard) and *P. maculosa* (Cady), met only the first criteria.

RESULTS

Hydrology

The Cady wetland was flooded throughout most of 2018 and 2019, while the Packard wetland had virtually no standing water during the study period (Tables 3, 4, $p<0.0001$ for between site comparison of control plots). There were no differences with compost addition at either site (Table 8). Water depths at the Cady wetland were higher in spring (6.7 – 13.3 cm) than summer or fall (Table 4). The Packard wetland was saturated in early spring and then dry for the remainder of the growing season (Table 4). Well measurements taken in spring (~28 cm below surface), summer (~43 cm below surface), and fall 2019 (~73 cm below surface) in the Cady wetland revealed a substantial decrease in water table depth as the growing season progressed.

Soil Properties

There were significant differences in soil properties between wetland sites and across treatment and seasons. As predicted by the variability in hydroperiod, MC was ~43% higher at Cady than Packard (control plots only, Table 3), and varied predictably with sampling date at both Cady ($p<0.0001$, Table 5) and Packard ($p=0.0022$, Table 6). At Cady, MC was significantly lower in summer 2018 (40%) compared to all measurements in 2019 (Table 5). At Packard, MC was higher in spring 2019 than summer or fall (Table 6). Compost significantly increased MC ($p<0.001$, Table 8), with an overall increase of ~14% at both sites.

There were no significant differences in OM between sites (control plots only, Table 3), but across all seasons, OM was 47% (Cady) and 20% (Packard) higher in compost treatments ($p<0.0001$ for both; Table 8), suggesting that treatments successfully increased soil organic matter. OM also varied seasonally in both Cady ($p<0.0001$, Table 5) and Packard ($p=0.0007$, Table 6). At Cady, OM was significantly lower in summer 2018 (19%) compared to all 2019 measurements (27-29% across compost and control plots). At Packard, OM was significantly lower in summer 2018 (15%) and spring 2019 (19%) compared to 22% in summer and fall 2019 (Table 6). There was a significant block effect in Packard. BD was ~11% lower at Cady than Packard (control plots only, $p=0.0003$, Table 3), and addition of compost decreased bulk density overall by ~40% at Cady and ~8% at Packard (Table 5, 6). We observed lowest BD at Cady in spring 2019 and in summer 2019 at Packard ($p<0.0001$ for both; Tables 5, 6). Although pH measurements were limited, observed values were significantly higher at Packard compared to Cady (7.8, $p<0.0001$; 7.3, $p<0.0001$, respectively), and with significant differences between sampling dates (Tables 5, 6), but no treatment effect (Table 8). Of note is the increase in pH at Cady between 2018 (7.0) and 2019 (7.5; Table 5, 6).

Soil Nutrients

Sites differed significantly in soil nutrients, with clear impacts of compost treatment. Soil %C was ~17% higher at Cady than Packard (control plots only, $p<0.0001$, Table 3) and was positively impacted by compost addition as expected (~50% and 28% increase, for Cady and Packard, respectively; $p<0.0001$, Table 8). Soil %C also varied seasonally at Cady only, with the lowest value in summer 2018 (~8.9; $p=0.0025$, Table 5) relative to all other values (13-17% across fall 2018 through fall 2019. Soil %N was slightly (~13%) higher at Cady than Packard ($p=0.17$, Table 3), and varied seasonally only at Cady ($p=0.0015$, Table 5). Percent N was highest in fall 2018 with a value of 1.1 compared to 0.6, 0.6, 0.7, and 0.7 in summer 2018, and spring, summer, and fall 2019, respectively. Compost increased %N by 53% (Cady) and 25% (Packard) ($p<0.0001$, Table 8). C:N molar ratio was 5% higher at Cady compared to Packard (control plots only, $p<0.0001$, Table 3) and 3-4% higher in compost plots at both sites ($p<0.001$ for both, Table 8). C:N molar ratio differed between seasons at both Cady ($p=0.0045$, Table 5) and Packard ($p=0.0075$, Table 6). At Cady, C:N was high in summer 2018 (~19) than the rest of 2018 and 2019. There were no differences in TP or IP between sites (Table 3), but both TP and

IP varied by season at both sites ($p<0.0001$, Table 5, 6). Greatest TP was found at Cady in fall 2019 (1.35 mg g^{-1}) and at Packard in spring 2019 (1.7 mg g^{-1}), with greatest IP at Cady in spring 2019 (0.84 mg g^{-1}) and at Packard in summer 2019 (0.91 mg g^{-1}). Compost addition did not increase TP, but did increase IP 21% and 20% at Cady and Packard, respectively ($p=0.04$, $p=0.005$, Table 8). Extractable NO_3^-, measured only in Summer and Fall 2018, was 97% higher at Packard than at Cady (control plots only, $p<0.0001$, Table 3). There were no between-site differences in extractable NH_4^+ ($p=0.2$, Table 3). Season had a significant impact on NO_3^- and NH_4^+ concentrations at both sites ($p<0.0001$, Table 5, 6). At Cady, NO_3^- and NH_4^+ were higher in summer 2018 (3.1 ug g^{-1}, 1147 ug g^{-1}, respectively) than fall 2018 (1.1 ug g^{-1}, 15 ug g^{-1}, respectively), with a similar pattern at Packard with summer 2018 (90 ug g^{-1}, 385 ug g^{-1}, respectively) greater than fall 2018 (24 ug g^{-1}, 7.3 ug g^{-1}, respectively). Compost addition had no effect on NO_3^- or NH_4^+ at either site (Table 8).

Total Vegetation Cover, Species Richness, and Shannon Diversity

Differences in emergent plant cover, *S*, and *H'* were observed between wetland sites and across seasons (Tables 3, 7), but not between compost treatments within a site (Table 8). Total plant cover was similar between sites when averaged across all seasons (Table 3), but this masks the interannual variability. Plant cover at Cady increased more than 14-fold from 2018 (~5%) to 2019 (~72%) as the vegetation community recovered from herbicide application (Table 7).Whereas at Packard, plant cover was more consistent between years, showing a seasonal low in fall (Table 7). Likewise, although *S* and *H'* were lower at Cady than Packard ($p=0.02$, $p<0.0001$), this was largely drivn by the low values at Cady in 2018 during the recovery of the plant community. *S* was 2.5 times greater, and *H'* 4 times greater in 2019 than 2018 at this site. There were no significant differences in % Cover, *S*, or *H'* at Packard between 2018 and 2019 ($p=0.7$, $p=0.45$, $p=0.25$, respectively). There were generally predictable seasonal changes in % cover, *S*, and *H'* at both sites ($p<0.0001$, Table 7), although these variables peaked in summer at Cady (2019 only) and in spring and summer at Packard. There was no impact of compost on *S* or *H'* (Table 8).

Potential Denitrification & Nitrogen Fixation

Rates of PDNF ranged from 0.05 to 0.74 ug N g^{-1} hr^{-1} at Cady and 0.08 to 0.49 ug N g^{-1} hr^{-1} at Packard (Figure 6). PDNF was significantly different between sites with rates ~50% higher at Cady than at Packard. At Cady, rates were similarly higher in 2018 and 2019 than in the fall 2017 measurement ($p<0.0001$; Table 8), while at Packard, fall 2017 and spring 2019 were lower than all summer measurements ($p=0.0021$, Table 8). Compost addition substantially increased PDNF at both sites, with increases of 56% ($p=0.0001$) and 47% ($p=0.0003$) in compost relative to control plots at Cady and Packard, respectively.

Soil and Plant-mediated N_2O Fluxes

N_2O flux associated with only soil microbial processes was about 30% higher at the Packard marsh, but overall the difference was not significant because of substantial spatial variability ($p=0.06$; Table 3). Soil N_2O fluxes measured at Cady (overall range = -0.005 - 0.047 mg N_2O-N m^{-2} hr^{-1}) and Packard (overall range = 0.008 - 0.059 mg N_2O-N m^{-2} hr^{-1}, (Figure 7). Fluxes varied significantly across seasons at both sites (Cady $p<0.0001$, Table 5, Packard $p=0.0019$, Table 6) with generally lower values in fall relative to other seasons. Soil N_2O fluxes, measured to specifically determine the effect of precipitation in fall 2019 apart from main study collections, were not significantly affected by rainfall (~16 mm in the prior 24 hr) at Cady ($p=0.78$) or Packard ($p=0.62$, Table A2). Before rainfall, rates were -0.001 and 0.008 mg N_2O-N $m^{-2}hr^{-1}$ at Cady and 0.007 and 0.008 mg N_2O-N $m^{-2}hr^{-1}$ at Packard (compost, control respectively). After rainfall, measurements ranged from 0.01 and -0.005 at Cady and 0.010 and 0.013 mg N_2O-N $m^{-2}hr^{-1}$ at Packard (compost, control, respectively).

N_2O fluxes measured in the chambers that enclosed both plants and soil) were highly variable and not significantly different between sites ($p=0.7$, Table 3) or with compost treatment ($p=0.6$). The rates were generally similar in magnitude to those measured in soil-only chambers. There were no significant differences between light and dark ecosystem N_2O fluxes at either site (Table A1). Ecosystem N_2O fluxes ranged from -0.03 to 0.06 mg N_2O-N m^{-2} hr^{-1} at Cady and -0.05 to 0.048 mg N_2O-N m^{-2} hr^{-1} at Packard and varied significantly across seasons at both sites (Cady $p=0.0045$, Packard $p<0.0001$, Figure 8).

Generalized Regression Analysis

Generalized regression analysis showed OM and WD as significant predictors of PDNF (Table 7). BD (r=-0.4, p=0.01), OM (r=0.3, p=0.04), %C (r=0.4, p=0.0015), precipitation (r=-0.4, p=0.003), and temperature (r=0.4, p=0.004) were correlated with PDNF at Packard. Generalized regression analysis showed %N, temperature, and TP as significant predictors at Packard (Table 7). A generalized regression across sites reveals %N ($p<0.0001$), temperature ($p<0.0001$), and C:N (p=0.9) as significant predictors of PDNF (Table 9).

Regression analysis across sites reveals precipitation (p=0.009) and OM (p=0.08) as significant predictors of soil N_2O fluxes (Table 9). Generalized regression analysis showed precipitation as the sole significant predictor of soil N_2O fluxes at Cady (Table 9). At Packard, MC (r=0.3, p=0.04), total cover (r=0.4, p=0.01), temperature (r=-0.5, $p<0.0001$), and H (r=0.4, p=0.01) were correlated with soil N_2O fluxes. Temperature and total cover were predictors of soil N_2O flux at Packard (Table 7).

Separate generalized regressions of *S. canadensis*, *P. arundinacea*, and *Polygonum pensylvanicum* % cover against %C, %N, and C:N ratios shows that *S. canadensis* % cover is a significant predictor of and negatively correlated with %N at Packard (AIC=-35, R^2=0.06, -0.006, F=3.1, p=0.02). No predictive relationships were found at the Cady wetland.

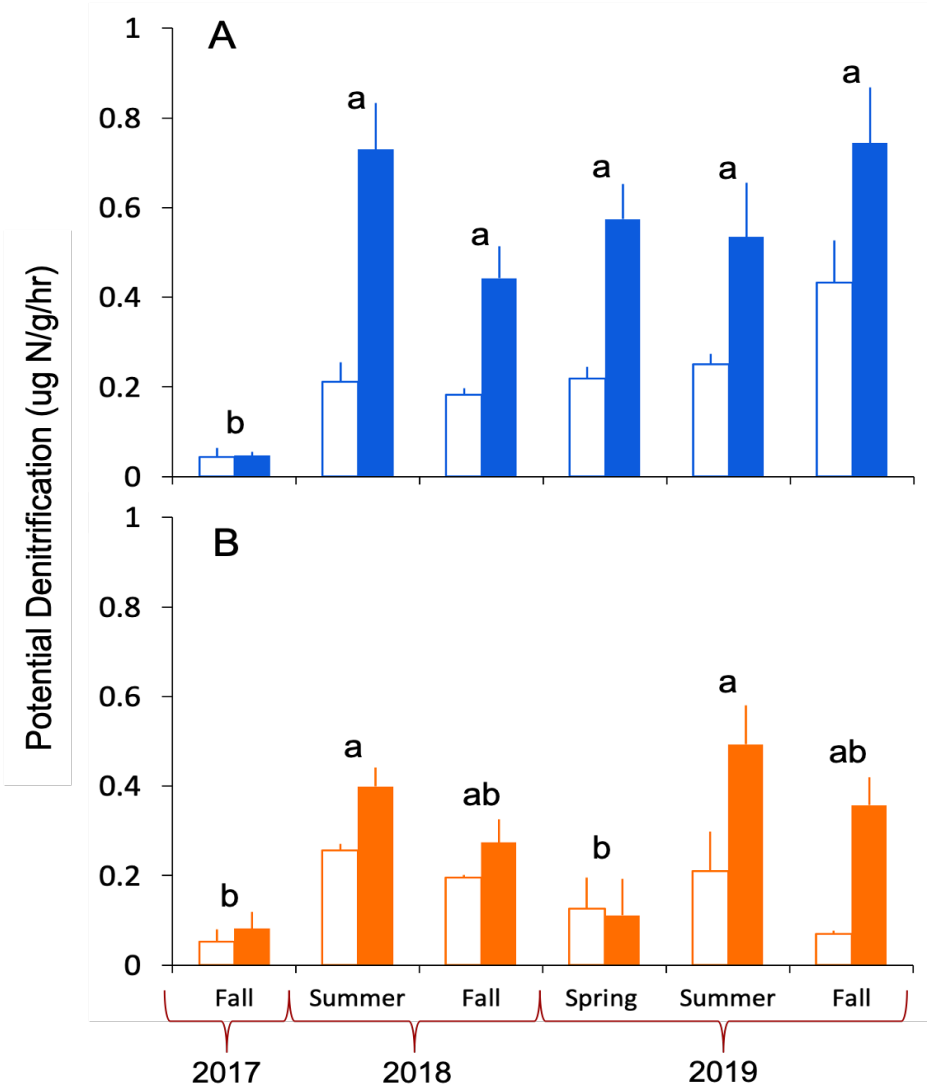

Figure 6. Potential denitrification, mean +/- SE (n=8-10). Unique letters above bars indicate significant differences among measurement dates at the (A) Cady and (B) Packard wetland sites.

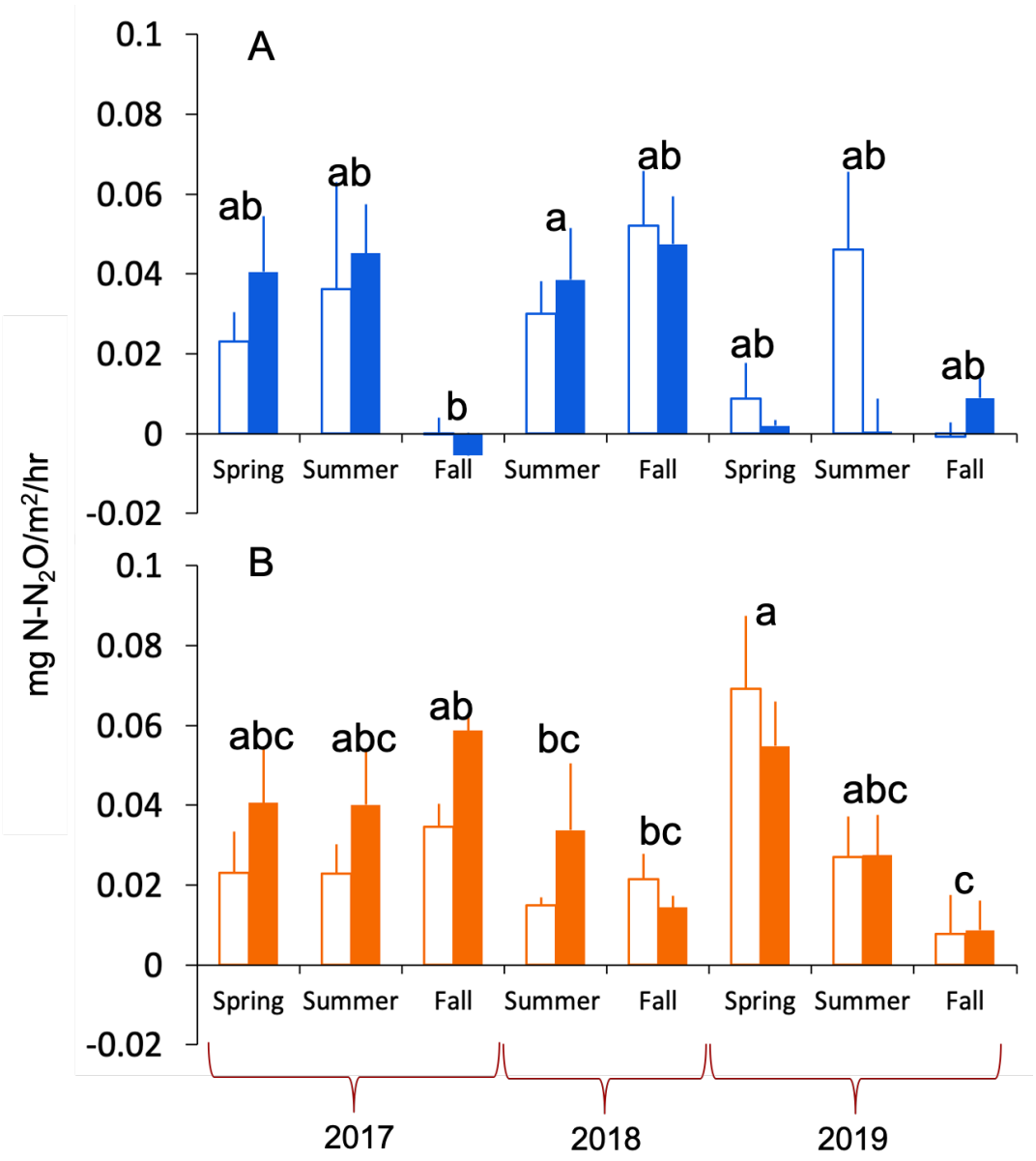

Figure 7. N_2O flux produced by soil microbial processes measured in small (~15 cm ID) chambers across seasons from 2017 to 2019, mean +/- SE (n=8) at Cady (A) and Packard (B). Unique letters above bars indicate significant differences among measurement dates.

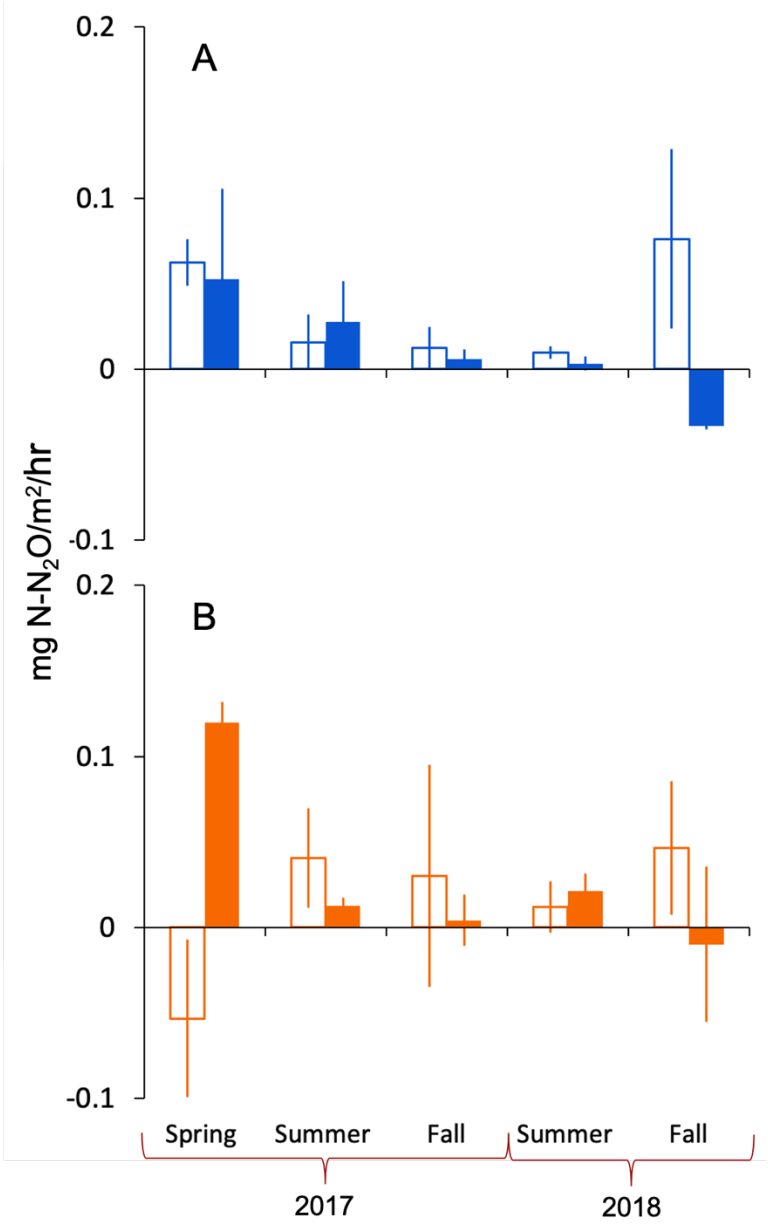

Figure 8. N_2O flux produced by ecosystem processes under light conditions (large chambers across seasons from 2017 to 2018, mean +/- SE [n=4]). Cady (A) and Packard (B). There were no differences among measurement dates.

Table 3. Site comparison of factors using only control treatments. Mean ± SE water depth (WD, cm), moisture content (MC, %), organic matter (OM, %), bulk density (BD, g cm^{-3}), pH, percent carbon (%C), percent nitrogen (%N), C:N (molar), total phosphorus (TP, mg g^{-1}), total inorganic phosphorus (IP, mg g^{-1}), NO_3^- (ug g^{-1}), NH_4^+ (ug g^{-1}), percent plant cover (% Cover), *S* (species richness), and *H'* (Shannon diversity index) measured at the Cady and Packard wetlands over the course of the study. Results of one-way ANOVA or Kruskal-Wallace tests on differences in metrics between sites are included. Stars next to values in the F statistic column denote Z values for Kruskal-Wallace tests conducted on data that did not meet the assumptions for the parametric test.

	Cady	Packard	df	F	p
WD	4.2 ± 0.50	0 ± 0	1,55	26	**<0.0001**
MC	50 ± 2.0	31 ± 1.62	1,90	123	**<0.0001**
OM	24 ± 0.90	18 ± 0.64	1,98	0.7	0.4
BD	0.5 ± 0.03	0.6 ± 0.02	1,80	14	**0.0003**
pH	7.3 ± 0.10	8 ± 0.05	1,40	40	**<0.0001**
%C	14 ± 0.90	9 ± 0.44	1,94	19	**<0.0001**
%N	0.9 ± 0.10	0.6 ± 0.07	1,94	1.9	0.17
C:N	18.1 ± 0.30	17.6 ± 0.20	1,94	30	**<0.0001**
TP	0.9 ± 0.04	0.90 ± 0.03	1,98	0.2*	0.9
IP	0.5 ± 0.03	0.58 ± 0.03	1,98	2.4*	**0.02**
NO_3^-	2 ± 0.20	59 ± 4.40	1,72	81	**<0.0001**
NH_4^+	220 ± 55	218 ± 53	1,74	11	0.2
% Cover	40 ± 0.61	33 ± 0.74	1,55	1.1	0.3
S	2.2 ± 0.20	3 ± 0.23	1,55	7.1	**0.01**
H	0.5 ± 0.10	0.8 ± 0.08	1,55	7.2	**0.01**
PDNF	0.3 ± 0.04	0.2 ± 0.03	1, 107	5.7	**0.02**
Soil N_2O	0.02 ± 0.01	0.03 ± 0.01	1, 127	0.2	0.6
Ecosystem N_2O	0.01 ± 0.02	0.002 ± 0.03	1,36	0.2	0.7

Table 4. Mean ± SE water depth (WD, cm) measured at the Cady wetland over the course of the study. Letters denote significant seasonal differences. Stars next to the variable name denote significant differences between treatments.

		Cady Wetland					
		2018			2019		
		spring	summer	fall	spring	summer	fall
WD	CTL *	9.7 ± 0.8^{a}	0 ± 0^{c}	2.6 ± 0.3^{bc}	13.3 ± 0.9^{a}	1.4 ± 0.6^{bc}	5.5 ± 0.6^{b}
	CMP	6.7 ± 0.7^{a}	0 ± 0^{c}	1.3 ± 0.3^{bc}	7.4 ± 0.7^{a}	0.6 ± 0.4^{bc}	1.4 ± 0.3^{b}

Table 5. Moisture content (MC, %), organic matter (OM, %), bulk density (BD, g cm^{-3}), pH, percent carbon (%C), percent nitrogen (%N), C:N (molar), total phosphorus (TP, mg g^{-1}), total inorganic phosphorus (IP, mg g^{-1}), NO_3^- (ug g^{-1}), and NH_4^+ (ug g^{-1}) measured at the Cady wetland in control (CTL) and compost (CMP) treatments over the course of the study. Letters denote significant seasonal differences. Stars next to the variable name denote significant differences between treatments.

		Cady Wetland				
		2018			2019	
		summer	fall	spring	summer	fall
MC	CTL *	43 ± 2.1^{c}	-	60 ± 2.4^{a}	48 ± 2^{b}	49 ± 1.6^{b}
	CMP	38 ± 1.4^{c}	-	70 ± 2.4^{a}	61 ± 2^{b}	63 ± 1.6^{b}
OM	CTL *	17 ± 1.9^{b}	-	19 ± 1.8^{a}	19 ± 1.1^{a}	18 ± 1.2^{a}
	CMP	21 ± 2.2^{b}	-	40 ± 2.2^{a}	36 ± 2.3^{a}	40 ± 1.1^{a}
BD	CTL *	0.6 ± 0.02^{a}	-	0.4 ± 0.02^{b}	0.6 ± 0.04^{a}	0.6 ± 0.03^{a}
	CMP	0.5 ± 0.03^{a}	-	0.2 ± 0.02^{b}	0.3 ± 0.04^{a}	0.3 ± 0.01^{a}
pH	CTL	6.9 ± 0.03^{a}	-	-	-	7.4 ± 0.08^{b}
	CMP	7.1 ± 0.09^{a}	-	-	-	7.5 ± 0.13^{b}
%C	CTL *	8.6 ± 0.5^{b}	11 ± 0.7^{a}	10 ± 0.5^{a}	8.1 ± 0.2^{a}	9.1 ± 0.3^{a}
	CMP	9.1 ± 0.5^{b}	20 ± 1.7^{a}	23 ± 1.7^{a}	18.1 ± 1.6^{a}	24 ± 0.8^{a}
%N	CTL *	0.5 ± 0.03^{b}	0.7 ± 0.04^{a}	0.6 ± 0.04^{a}	0.6 ± 0.02^{ab}	0.6 ± 0.02^{a}
	CMP	0.6 ± 0.03^{b}	1.4 ± 0.1^{a}	1.6 ± 0.1^{a}	1.2 ± 0.1^{ab}	1.6 ± 0.1^{a}
C:N	CTL *	18.9 ± 0.6^{a}	17.4 ± 0.7^{b}	18.2 ± 0.2^{b}	18.0 ± 0.1^{b}	18.1 ± 0.2^{b}
	CMP	18.8 ± 0.2^{a}	18.4 ± 1.4^{b}	17.0 ± 0.2^{b}	17.5 ± 0.3^{b}	17.2 ± 0.2^{b}
TP	CTL *	0.46 ± 0.01^{b}	0.61 ± 0.03^{b}	1.12 ± 0.02^{a}	1.2 ± 0.07^{a}	1.14 ± 0.05^{a}
	CMP	0.51 ± 0.03^{b}	0.68 ± 0.05^{b}	1.2 ± 0.02^{a}	1.15 ± 0.07^{a}	1.35 ± 0.07^{a}
IP	CTL *	0.30 ± 0.02^{b}	0.31 ± 0.03^{b}	0.71 ± 0.01^{a}	0.73 ± 0.07^{a}	0.63 ± 0.04^{a}
	CMP	0.29 ± 0.006^{b}	0.40 ± 0.03^{b}	0.84 ± 0.02^{a}	0.5 ± 0.01^{a}	0.81 ± 0.07^{a}
NO_3^-	CTL	2.9 ± 0.3^{a}	1 ± 0.01^{b}	-	-	-
	CMP	3.2 ± 0.5^{a}	1.1 ± 0.1^{b}	-	-	-
NH_4^+	CTL	428 ± 108^{a}	12 ± 1^{b}	-	-	-
	CMP	1866 ± 129^{a}	17 ± 1.4^{b}	-	-	-

Table 6. Moisture content (MC, %), organic matter (OM, %), bulk density (BD, g cm^{-3}), pH, percent carbon (%C), percent nitrogen (%N), C:N (molar), total phosphorus (TP, mg g^{-1}), total inorganic phosphorus (IP, mg g^{-1}), NO_3^- (ug g^{-1}), and NH_4^+ (ug g^{-1}) measured in control (CTL) and compost (CMP) treatments over the course of the study. Letters denote significant seasonal differences. Stars next to the variable name denote significant differences between treatments.

Packard Wetland						
		2018		2019		
		summer	fall	spring	summer	fall
MC	CTL *	31 ± 2.2^{ab}	-	32 ± 0.9^{a}	25 ± 1.7^{ab}	25 ± 0.7^{b}
	CMP	34 ± 1.8^{ab}	-	36 ± 1^{a}	33 ± 1.4^{ab}	28 ± 1.7^{b}
OM	CTL *	14 ± 0.8^{b}	-	16 ± 1^{ab}	16 ± 1^{a}	17 ± 1^{a}
	CMP	16 ± 0.7^{b}	-	22 ± 1^{ab}	28 ± 2^{a}	26 ± 2^{a}
BD	CTL *	0.6 ± 0.01^{a}	-	0.7 ± 0.02^{a}	0.6 ± 0.01^{b}	0.7 ± 0.02^{a}
	CMP	0.7 ± 0.04^{a}	-	0.5 ± 0.02^{a}	0.4 ± 0.01^{b}	0.6 ± 0.03^{a}
pH	CTL	7.7 ± 0.05^{a}	-	-	-	7.7 ± 0.04^{b}
	CMP	7.7 ± 0.05^{a}	-	-	-	7.5 ± 0.06^{b}
%C	CTL *	7.2 ± 0.4	8 ± 0.5	9 ± 0.5	8 ± 0.5	7 ± 0.6
	CMP	8.2 ± 0.4	10 ± 0.1	11 ± 0.5	14 ± 0.6	12 ± 0.6
%N	CTL *	0.5 ± 0.02^{b}	0.5 ± 0.01^{b}	0.6 ± 0.4^{ab}	0.6 ± 0.03^{a}	0.5 ± 0.03^{ab}
	CMP	0.5 ± 0.02^{b}	0.6 ± 0.04^{b}	0.7 ± 0.3^{ab}	0.9 ± 0.03^{a}	0.8 ± 0.04^{ab}
C:N	CTL *	18 ± 0.2^{ab}	20 ± 0.1^{a}	17 ± 0.1^{b}	17 ± 0.3^{b}	16 ± 0.4^{b}
	CMP	18 ± 0.3^{ab}	19 ± 0.2^{a}	17 ± 0.2^{b}	18 ± 0.1^{b}	17 ± 0.2^{b}
TP	CTL	0.52 ± 0.01^{b}	0.58 ± 0.03^{b}	1.12 ± 0.05^{a}	1.04 ± 0.04^{a}	1.25 ± 0.04^{a}
	CMP	0.56 ± 0.01^{b}	0.62 ± 0.02^{b}	1.7 ± 0.1^{a}	1.3 ± 0.05^{a}	1.22 ± 0.01^{a}
IP	CTL *	0.31 ± 0.007^{b}	0.29 ± 0.02^{b}	0.83 ± 0.06^{a}	0.75 ± 0.03^{a}	0.73 ± 0.02^{a}
	CMP	0.33 ± 0.008^{b}	0.35 ± 0.01^{b}	0.59 ± 0.01^{a}	0.91 ± 0.006^{a}	0.89 ± 0.01^{a}
NO_3^-	CTL	94 ± 6^{a}	25 ± 2.5^{b}	-	-	-
	CMP	83 ± 6^{a}	23 ± 0.1^{b}	-	-	-
NH_4^+	CTL	428 ± 104^{a}	7.7 ± 1.4^{b}	-	-	-
	CMP	342 ± 49^{a}	6.8 ± 1.2^{b}	-	-	-

Table 7. Percent plant cover (% Cover), S (species richness), and Shannon diversity index (H) measured at the Packard wetland measured in control (CTL) and compost (CMP) treatments over the course of the study. Letters denote significant seasonal differences. Stars next to the variable name denote significant differences between treatments.

			2018			2019		
			spring	summer	fall	spring	summer	fall
Cady	% Cover	CTL	3 ± 0.2^{b}	3 ± 0.3^{b}	8 ± 0.5^{ab}	21 ± 0.1^{ab}	99 ± 0.1^{a}	95 ± 1.1^{ab}
		CMP	1.7 ± 0.2^{b}	3 ± 0.3^{b}	12 ± 0.7^{ab}	29 ± 0.7^{ab}	131 ± 1^{a}	71 ± 0.8^{ab}
	S	CTL	1.1 ± 0.1^{cd}	1.0 ± 0.1^{d}	1.7 ± 0.2^{bc}	2.9 ± 0.2^{ab}	4.0 ± 0.2^{a}	3.3 ± 0.2^{a}
		CMP	0.9 ± 0.1^{cd}	0.7 ± 0.1^{d}	2.0 ± 0.3^{bc}	2.7 ± 0.2^{ab}	3.6 ± 0.2^{a}	3.0 ± 0.2^{a}
	H	CTL	0.1 ± 0.04^{cd}	0.1 ± 0.03^{d}	0.4 ± 0.08^{bc}	0.7 ± 0.01^{ab}	0.9 ± 0.1^{a}	0.9 ± 0.1^{a}
		CMP	0.1 ± 0.04^{cd}	0.1 ± 0.04^{d}	0.4 ± 0.08^{bc}	0.6 ± 0.01^{ab}	0.7 ± 0.1^{a}	0.7 ± 0.1^{a}
Packarc	% Cover	CTL	26 ± 0.7^{bc}	38 ± 0.9^{ab}	9.9 - 0.8^{c}	38.6 ± 0.7^{a}	38.6 ± 0.8^{a}	16.7 ± 0.5^{c}
		CMP	25 ± 0.6^{bc}	39 ± 0.9^{ab}	4.6 - 0.61^{c}	48.8 ± 0.7^{a}	45.3 ± 0.8^{a}	13.8 ± 0.5^{c}
	S	CTL	3.4 ± 0.2^{a}	3 ± 0.3^{ab}	0.83 - 0.29^{c}	3.6 ± 0.2^{a}	3.5 ± 0.2^{a}	2.4 ± 0.2^{b}
		CMP	4.2 ± 0.2^{a}	2.8 ± 0.3^{ab}	0.7 - 0.32^{c}	3.9 ± 0.2^{a}	3.3 ± 0.2^{a}	2 ± 0.2^{b}
	H	CTL	1 ± 0.1^{a}	0.7 ± 0.1^{ab}	0.26 - 0.01^{c}	0.9 ± 0.1^{a}	0.8 ± 0.1^{a}	0.5 ± 0.1^{b}
		CMP	0.8 ± 0.1^{a}	0.8 ± 0.1^{ab}	0.2 - 0.01^{c}	0.9 ± 0.1^{a}	0.8 ± 0.1^{a}	0.5 ± 0.1^{b}

Table 8. Results of two way ANOVA or Kruskall Wallace tests on the effects of treatment, season, and their interaction on water depth (WD, cm), moisture content (MC, %), organic matter (OM, %), bulk density (BD, g cm^{-3}), pH, percent carbon (%C), percent nitrogen (%N), C:N (molar), total phosphorus (TP, mg g^{-1}), total inorganic phosphorus (IP, mg g^{-1}), NO_3^- (ug g^{-1}), NH_4^+ (ug g^{-1}), percent plant cover (% Cover), S (species richness), and Shannon diversity index (H), potential denitrification (PDNF), soil N_2O fluxes, ecosystem N_2O fluxes at Cady (C) and Packard (P) throughout the length of the study. Significant p-values are bolded. Stars indicate where significant block effects were observed.

		treatment		season		trt x sea		
		F, df---X	p	F, df--- X	p	F, df	p	
WD	C	Z_1 =1.6	0.11	X_5=48.3	**<0.0001**			
	P	-	-	-	-	-	-	
MC	C	$F_{1,79}$ =48.0	**<0.0001**	$F_{3,79}$ =47.0	**<0.0001**	$F_{3,79}$ = 1.9	0.14	
	P	$F_{1,96}$ =14.2	**0.0003**	$F_{4,94}$ =4.5	**0.0022**	$F_{4,94}$ =0.42	0.79	
OM	C	Z_1 =4.4	**<0.0001**	X_2 =47.4	**<0.0001**			
	P	Z_1 =5.6	**<0.0001**	X_4 =12.5	**0.0007**			***
BD	C	Z_1 =4.9	**<0.0001**	X_3 =19.6	**<0.0001**			
	P	$F_{1,78}$ =21.6	**<0.0001**	$F_{3,76}$ =10.4	**<0.0001**	$F_{3,76}$ =0.07	0.98	
pH	C	Z_1 =1.6	0.19	X_1 =3.9	**<0.0001**			
	P	$F_{1,38}$ =3.5	0.07	$F_{1,38}$ =13.0	**0.001**	$F_{1,38}$ =1.6	0.21	
%C	C	Z_1 =6.2	**<0.0001**	X_4 =16.4	**0.003**			
	P	$F_{1,85}$ =45.9	**<0.0001**	$F_{2,85}$ =1.4	0.24	$F_{2,85}$ =1.3	0.27	
%N	C	Z_1 =6.0	**<0.0001**	X_4= 13.3	**0.002**			
	P	Z_1 =4.5	**<0.0001**	X_2= 1.97	0.37			
C:N	C	Z_1 =4.5	**<0.0001**	X_5 =14.5	**0.005**			
	P	Z_1 =3.6	**0.0004**	X_4 =13.9	**0.008**			
TP	C	Z_1 =1.2	0.22	X_4 =72.1	**<0.0001**			
	P	Z_1 =1.7	0.10	X_4 =72.0	**<0.0001**			
IP	C	Z_1 =2.1	**0.040**	X_4 =66.6	**<0.0001**			
	P	Z_1 =2.8	**0.005**	X_4 =71.0	**<0.0001**			
NO_3^-	C	$F_{1,36}$ =0.31	0.58	$F_{1,36}$ =66	**<0.0001**	$F_{1,36}$ =0.14	0.71	
	P	Z_1 =0.32	0.74	Z_1 =5.0	**<0.0001**			
NH_4^+	C	Z_1 =1.3	0.20	Z_1 =5.3	**<0.0001**			
	P	Z_1 =0.24	0.81	Z_1 =5.1	**<0.0001**			
% Cover	C	Z_1 =0.39	0.70	X_5 =51.7	**<0.0001**			
	P	$F_{1,42}$ =0.78	0.38	$F_{4,42}$ =12.8	**<0.0001**	$F_{4,42}$ =0.65	0.63	
S	C	Z_1 =0.84	0.40	X_5 =39.7	**<0.0001**			
	P	$F_{1,42}$ =0.18	0.68	$F_{4,42}$ =7.6	**0.0002**	$F_{4,42}$ = 0.97	0.43	
H	C	Z_1 =0.91	0.36	X_5 =40.7	**<0.0001**			
	P	$F_{1,46}$ =0.39	0.84	$F_{4,46}$ =6.5	**0.0005**	$F_{4,46}$ = 0.86	0.43	
PDNF	C	$F_{1,94}$ =31.4	**<0.0001**	$F_{5,94}$ =12.1	**<0.0001**	$F_{5,94}$ =0.14	0.22	***
	P	Z_1 =3.6	**0.0003**	X_5 =18.6	**0.0021**			
Soil N2O	C	Z_1 = 0	1.0	X_7 =44.2	**<0.0001**			
	P	Z_1 =0.34	0.73	X_7 =22.7	**0.002**			
Ecosystem N2O	C	$F_{1,15}$ =0.48	0.64	$F_{2,15}$ = 13.1	**0.001**	$F_{2,15}$ =2.2	0.15	
	P	$F_{1,24}$ =0.24	0.63	$F_{3,24}$ =0.30	**<0.0001**	$F_{3,24}$ =2.6	0.08	

Table 9. Summarized results of generalized regressions with correlation coefficients, F values, and *p* values for the effect of each significant predictor. PDNF and soil N_2O models displayed for an over-arching model across sites and then for each site individually, with AICc and R^2 values.

	Model	AICc	R^2	Variable	Coefficient	F	*p*
Combined	PDNF	-23	0.43	%N	0.40	53.4	**<0.0001**
				Temperature	0.02	16.3	**0.0001**
				C:N	0.03	3	0.9
	Soil N_2O	-304	0.11	Precipitation	0.0007	7	**0.009**
				OM	-0.0009	3.2	0.08
Cady	PDNF	12	0.34	OM	0.01	14.3	**0.0004**
				Water Depth	-0.02	3.4	0.07
	Soil N_2O	-127	0.27	Precipitation	0.001	8.3	**0.006**
Packard	PDNF	-52	0.52	%N	0.70	26.9	**<0.0001**
				Temperature	0.02	12.7	**0.0009**
				TP	-0.2	6.6	**0.01**
	Soil N_2O	-232	0.38	Temperature	-0.002	18.8	**<0.0001**
				Total Cover	0.0005	6	**0.02**

DISCUSSION

Overview

Large-scale compost addition shows potential as a management tool to enhance restored wetland function and N removal in wetlands with varying hydroperiod, without substantially increasing N fixation or undesirable N_2O emissions. Enhanced soil C and moisture content associated with the addition were likely the driving factors. However, the magnitude of the response is driven by site-specific hydrology, underscoring the importance of management of both soil properties and water levels in created wetlands.

Site Differences

The fundamental differences in environmental conditions between the two sites in this study are the probably drivers of overall N cycling and the response to experimental treatments (Table 3). Hydroperiod variation, evidenced by higher water levels and moisture content at Cady, likely influenced overall C availability and lability by limiting decomposition at this site (Song et. al. 2014). At the same time, inorganic N form also varied, with NO_3^- levels 30 times higher at Packard and NH_4^+ levels >200 times greater at Cady, perhaps due to enhanced nitrification propagated by a more aerobic environment at Packard (Austin et. al. 2019). These differences in C and N quality and quantity, ultimately driven by hydrology, likely played a role in the response to compost addition.

The dryness of Packard may partially explain the higher species richness and *H*' present compared to Cady, as these conditions allow for a more diverse range of both grassland and wetland species. However, herbicide treatments at the end of the 2017 growing season at Cady effectively reset plant community development with very low cover and diversity in 2018 that eventually recovered in 2019. Vegetation community composition may also an important role in determining denitrification by altering soil C quantity, lability, and nutrient availability (Sirihedvin and Gray 2006, Hopfensperger et. al. 2009, Lishawa et. al. 2014, Zhang et. al. 2017, Yang et. al. 2019). While community diversity and plant cover did not appear to influence measured PDNF, trends arose from individual plants species. Among the most prominent plant species identified at both sites, only *S. candensis* cover emerged as a (negative) predictor of %N at Packard. In a study by Ye et. al. (2019), *S. candensis*, a pernicious invasive plant in the coastal grasslands of East China, accelerated macronutrient cycling via increases in aboveground productivity and nutrient accumulation in biomass. High nutrient uptake by *S. candensis* may explain the negative correlation observed in this study, as patchy increases in cover may have been linked to small-scale decreases in soil N. Further, while we don't have soil data from fall 2017 to support this contention, plant cover at Cady was nearly 100% *P. arundinacea* (pers. obs.). The capacity of this species for rapid uptake of nutrients (Stefanowicz et. al. 2018) may have lowered soil N (and in turn PDNF). From 2018 forward, nether *P. arundinacea* or *P.*

pennsylvanicum emerged as a predictor of soil characteristics, perhaps due to the high temporal and spatial variability of both species.

Effect of Compost Addition on Soil Characteristics

Large-scale addition of municipal leaf litter compost was effective at improving soil characteristics associated with known dominant drivers of denitrification at both sites. The additional organic matter enhanced moisture content and decreased bulk density, likely creating additional heterogeneity within the soil matrix for microscale oxic/anoxic interfaces. Total C, N, and inorganic P availability all increased, but the site-specific impact on C:N suggests a slightly more refractory C pool with compost addition at Packard and more labile at Cady. This difference in soil response may be due to the initial conditions at each site, where the compost C:N was similar to the initial soil raio at Cady and slightly higher at Packard. Addition did not have an effect on extractable NO_3^- or NH_4^+, suggesting that mineralized N was rapidly consumed by plants and microbes. Plant cover, *S*, and *H'* were not affected, suggesting that the quantity of compost added was not sufficient to see changes in community structure over the shorter term of the study. Interestingly, over the course of the experiment, soil C and N continue to increase at Cady, showing the impact of repeated additions of organic matter; whereas at Packard, the difference between treatments remains, but all additional N and C is consumed leading to no cumulative impact of the addition. This indicates that for effective long term soil enhancement, the hydroperiod must be considered and that at drier sites, greater C addition may be needed.

Controls on Nitrogen Fixation and Denitrification

Our observed rates of N fixation were sufficiently low that they could not be quantified with the methods used. This is in contrast to Eckardt and Biesboer (1988), who found that N-fixation can be a significant input of N to freshwater wetlands (up to 11 kg ha^{-1} yr^{-1}). Previous work in salt marshes has also shown a substantial increase in N fixation with C addition (from 7.7 umol N_2 m^{-2} h^{-1} preaddition to 240 umol N_2 m^{-2} h-1; Murphy et al. 2017). These contrasting results suggests that in such young wetlands as those in the present study, that there may be numerous factors contributing to such low rates. Heterotrophic N fix may have been limited by C availability at these sites (Cohen et. al. 2009), while autotrophic N fix may have been limited by light availability (Moseman et. al. 2009). It's of course also possible that high N availability may

have significantly limited overall N-fixation (Vitousek et. al. 2002). At Packard, low N-fix may also be due to the dryness of the site and resulting oxic soil matrix, as nitrogen fixation requires anaerobic conditions (Šantrůčková et. al. 2010). Nonetheless, N fixation in the soil is not a substantial source of N to these young wetlands.

The range of PDNF rates observed in this study (0.1-1.0 ug N/g/hr) were within previously reported ranges for created freshwater emergent wetlands (0.05-0.1 to 5.8-6.2 ug N/g/hr, Ballentine et. al. 2014, Salk et. al. 2018, respectively), and overall varied predictably with season, which was a significant predictor across sites (Table 7). The higher rates at Cady than Packard, roughly 50%, suggest that the inherent differences in soil characteristics and other environmental conditions determine baseline denitrification along with the response to compost addition, which was also somewhat greater at Cady (56% enhancement versus 47% at Packard). Interestingly, across sites, C:N emerged as a positive predictor of PDNF although not a significant one. This may be related to the inter-site differences in C:N, with the greater rates associated with Cady, where PDNF was greater in spite of a more refractory C pool. This is supported by the positive role of soil %N in predicting PDNF: enhanced N (and C) availability promoted overall PDNF. Not surprisingly, temperature was also a strong predictor of PDNF, illustrating the seasonal dependence of microbial processes (Song et. al. 2010). In addition to the overall influence of season on heterotrophic activity, the potential for exudation of labile C in the root zone by plants is greatest during the summer when plant growth is at its peak (Moore et. al. 2015). However, within each site, slightly different patterns emerged, suggesting site-specific drivers.

Substantial C limitation of PDNF at the Cady wetland is illustrated by the highly significant response to compost addition and the emergence of OM as the primary predictor variable. Although soil C levels at this site were already significantly higher than at Packard, compost addition elicited higher rates of N removal suggesting that neither N availability or excess oxygen (soil dryness) were limiting. Further, previous research has shown that C source is critically important towards effective promotion of denitrification (Sirivedhin and Gray 2006, Ballentine et. al. 2014, Lishawa et. al. 2014, Zhang et. al. 2017) and it is assumed that some of the C added via leaf litter compost was highly labile and consumption of this more available

fraction fostered enhanced heterotrophic activity. In such young wetlands, where soil C stocks are low, it is clear that enhancement of soil quality by organic matter addition promotes development of ecosystem function. Further, the application of herbicide is likely to have substantially impacted denitrification, as probably illustrated by the substantial increase in PDNF between fall 2017 and rates in all subsequent seasons, although we do not have soil data across this period and further study is required.

Interestingly, however, with regard to hydrology, it appears that perhaps at Cady, at some times of the year and in some of the experimental blocks, the higher water levels may inhibit PDNF. This is perhaps because under high water conditions oxygen diffusion through the water column is decreased and nitrification becomes limited by increasing anoxia (Austin et. al. 2019). This, in turn, limits the availability of NO_3^- for denitrification and eliminates the potential for coupled nitrification-denitrification.

In contrast, a different set of predictive drivers appeared at the drier Packard wetlands. Seasonal shifts were important, with higher rates in summer, especially with compost addition. In this case, it is likely that the N supplied by the compost was key in facilitating the enhanced PDNF under experimental conditions. While NO_3^- availability at this site was typically higher than at the wetter site, likely because of oxidizing conditions that promote nitrification, rates increased over time in concert with overall higher soil N. Along with the higher N, the soil moisture was increased in the compost treatments, perhaps further promoting denitrification in the wetter microzones where anaerobic metabolism may proceed. TP as a negative predictor is contrary to a number of studies showing limitation of denitrification by P availability (White and Reddy 1999, Sandraweshar et. al. 2003). At both sites, TP and IP increased over the course of the study, perhaps as a result of further sequestration of P in soil as the ecosystem matured.

Controls on N_2O Fluxes

Soil and ecosystem N_2O fluxes in the present study - -0.0007 to 0.07 mg N $m^{-2}hr^{-1}$ for soil-only fluxes and 0.002 to 0.05 mg N $m^{-2}hr^{-1}$ for ecosystem flux measurements - were within the range previously reported in coastal wetlands and in microcosms (Lyu et. al. 2017, Yang et. al. 2019). The high variability of the ecosystem fluxes, even within a site for a single season, precluded

identification of any distinct spatial or temporal patterns. But overall, the range of values is similar to that measured for soil-only fluxes, suggesting a minor role of plant-mediated transport of N_2O.

Across sites, precipitation and OM were the main drivers of soil N_2O fluxes. A fluctuating hydroperiod presented by high precipitation may explain the positive correlation between precipitation and N_2O fluxes, as denitrification can be interrupted due to sudden exposure to aerobic conditions, resulting in enhanced N_2O release (Knowles 1996, Kampschreur et. al. 2009). While some studies have reported decreases in N_2O production as a result of added C amendments such as sodium acetate (Lyu et. al. 2017, Yang et. al. 2019), we observed no impact of leaf litter compost addition on soil or ecosystem N_2O fluxes. The negative correlation between OM and N_2O fluxes may be due to the relatively high C:N of the leaf litter compost (~18), as other studies have found that low C:N (ex. 4, 7) is linked to increases in N_2O production in created wetlands, mainly due to inhibition of N_2O nitrogenase activity (Wang et. al. 2014, Lyu et. al. 2017, Yang et. al. 2019). This suggests that the use of leaf litter compost to enhance soil development, and subsequently denitrification, does not have the concomitant side effect of increasing N_2O production.

Within individual sites, specific drivers of soil N_2O fluxes differed. At Cady, precipitation was the sole significant driver of N_2O fluxes suggesting that recent rain events may stimulate N_2O production. This wasn't, however, confirmed by the paired comparison of flux rates measured in fall 2019 after a moderately dry stretch (16 mm of rain over a 10 d period) and those measured immediately following a wetter stretch (24 mm of rain over a 10 d period with 16 mm occurring in the previous 24 hr). Thus, the longer term pattern suggests that it is the cumulative rainfall and impact on the hydroperiod that drives N_2O release. We did not see a similar relationship for PDNF at this site, indicating that precipitation may favor production of N_2O, but not denitrification. The positive correlation between precipitation and fluxes may be due to sudden aeration from the action of water droplets hitting the water column, disrupting denitrification and resulting in N_2O release. The negative relationship between temperature and N_2O may be driven by the higher observed fluxes in spring and fall, when reductions in activity of denitrifying microbial communities under cooler temperatures may occur (Sirihedvin and Gray 2006). As a

secondary driver, total cover was positively correlated with N_2O fluxes, perhaps due to aeration of the soil by increased rhizosphere presence that disrupted complete denitrification (Song et. al. 2014).

Effect of Compost Addition on Nitrogen Removal Efficiency

In partial agreement with our results, Yang et. al. (2019) found that addition of C sources could improve N removal while reducing N_2O emissions, in essence increasing complete denitrification. The efficiency of nitrogen removal may be calculated as N_2/N_2+N_2O, and provides a reasonable proxy for analysis of complete versus incomplete denitrification at these wetland sites. We do acknowledge, however, that the N_2O release is the actual measured rate in the field, while the PDNF represents only a potential rate under more or less optimal conditions in the laboratory. As such, the actual denitrification in the field may be somewhat lower. At Cady, the efficiency increased moderately, by ~6%, as a result of compost addition (df=1, F= 4.9, p=0.032), suggesting an increase in potential N removal while preventing an equivalent rise, if not a reduction, in N_2O emissions, effectively increasing complete denitrification.

In contrast, at the Packard wetland, where soil was consistently dry during the growing season, the N removal efficiency was not different with compost addition suggesting an increase in both potential N_2 and N_2O production (df=1, f=0.89, p=0.36). We suspect that the lack of saturation led to more frequently aerobic conditions and disruptions to complete denitrification (Knowles 1996, Kampschreur et. al. 2009), in spite of the greater availability of C. However, there was not a significant increase in N_2O with compost, likely due to the high spatial and temporal heterogeneity of these fluxes. As such, we suggest that the influence of compost in enhancing complete denitrification remains the dominant impact on the N cycle at this site.

Compost Addition as a Management Tool

The increase in N added via compost addition (~125 g N m^{-2}, per site, per year) was roughly similar to the subsequent increase in potential removal of N in the 10 cm of soil during the April-November growing season (compost minus control = 192 g N m^{-2} and 107 g N m^{-2} at Cady and Packard, respectively). The lower enhancement of PDNF at Packard is likely indicative of hydrological differences between sites, suggesting hydrology must also be managed to achieve

maximum N removal with C addition. However, the robust plant community at Packard throughout the study may also contribute to the lower microbial N removal by competing for the additional N. Thus, overall nutrient and C cycling was heightened by enhanced soil fertility, which may stimulate greater plant health and higher system throughput and community development.

The results of this study confirm that hydrology, C availability, and N availability are key drivers of denitrification in created wetlands and that management of these factors will aid in the establishment of robust nutrient removal services. Undetectable levels of N fixation may imply that the impact and contribution of soil microbial N fixation may be insignificant within the overall N budget in these very young wetlands. Leaf litter compost is readily available through many municipalities, and as such represents a viable, cheap solution to the problem of poor soil quality in newly created wetlands. The addition of compost as a management technique increased soil organic matter, % soil C, moisture content, and led to increased potential denitrification, without significantly increasing N_2O emissions. In general, these results provide insight into management techniques that maximize N removal capacity and ecosystem function of created freshwater wetlands.

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APPENDIX

Table A1. Results of paired t-test comparing means of light and dark ecosystem N_2O flux measurements, separated out by treatment and site.

		df	t	p
Cady	Control	18	-0.9	0.37
	Compost	19	1.7	0.11
Packard	Control	14	-0.3	0.74
	Compost	13	0.2	0.87

Table A2. Results of paired t-test comparing means of fall soil N_2O fluxes measurements and fall soil N_2O flux measurements taken after rainfall, separated out by site.

		df	t	p
Cady	Control	3	0.3	0.78
Packard	Control	3	-0.2	0.84

Table A3. % cover of *S. canadensis*, *P. arundinacea*, *P. maculosa*, and *P. pensylvanicum* measured in control (CTL) and compost (CMP) treatments over the course of the study at Cady and Packard wetlands.

			2018			2019		
			spring	summer	fall	spring	summer	fall
Cady	*S. canadensis*	CTL	0 ± 0	0.1 ± 0.3	0.43 ± 0.2	3.7 ± 1.0	0.85 ± 0.5	0 ± 0
		CMP	0.8 ± 0.3	1.2 ± 0.3	0.68 ± 0	8.9 ± 3.0	16.3 ± 4.7	2.75 ± 0.8
	P. arundinacea	CTL	0 ± 0	0 ± 0.1	0 ± 0.0	2.2 ± 1.1	3.25 ± 1.7	6.63 ± 2.0
		CMP	0 ± 0	0 ± 0.1	0 ± 0.0	3.35 ± 1.3	6.3 ± 1.8	12.5 ± 3.8
	P. maculosa	CTL	1.2 ± 0.8	0.7 ± 0.03	2.78 ± 0.3	8.61 ± 1.5	28.1 ± 3.5	12.9 ± 2.6
		CMP	0.5 ± 0.2	1.1 ± 0.04	2.18 ± 0.4	10.6 ± 1.3	24.5 ± 4.2	19.8 ± 4.3
Packard	*S. canadensis*	CTL	4 ± 1.3	5.1 ± 1.6	2.25 - 0.1	7.95 ± 2.7	15.7 ± 3.2	5.58 ± 1.0
		CMP	2.2 ± 0.5	6.5 ± 2.4	2.33 - 0.4	7.08 ± 2.1	14.1 ± 3.9	3.48 ± 0.95
	P. arundinacea	CTL	0.4 ± 1.0	0 ± 0	0.0 - 0	0.0 ± 0	0.0 ± 0	0 ± 0
		CMP	1.2 ± 0.2	0 ± 0	0.79 - 0.1	0.0 ± 0	0.38 ± 0.3	0.13 ± 0.1
	P. pensylvanicum	CTL	0 ± 0	2 ± 1.1	5.83 - 1.3	1.05 ± 0.6	0.0 ± 0	0.38 ± 0.3
		CMP	0 ± 0	0.9 ± 0.5	2.83 - 0.2	1.98 ± 1.5	0.0 ± 0	0.03 ± 0.03